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Risk assessment of pollen-mediated gene flow from *Ga1-m* field corn to dent-sterile *Ga1-s* popcorn

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Abstract

The popcorn industry in the United States is at risk of genetic contamination because it utilizes the *gametophyte factor 1* gene (*Ga1*) as a barrier against pollen-mediated gene flow (PMGF) from field corn (*Zea mays* L.). Popcorn with the *Ga1-s* allele accepts pollen only from *Ga1-s* corn, allowing for field corn and popcorn to be nearby without isolation. Germplasm is being introduced to the United States to increase field corn diversity that unknowingly contains the *Ga1-m* allele, which can overcome *Ga1-s* selectivity and pollinate popcorn. The risk to the popcorn industry has been underassessed. Experiments were conducted to model the frequency of PMGF from *Ga1-m* field corn to *Ga1-s* popcorn under field conditions and to evaluate the role of wind speed and direction using a concentric donor–receptor design in 2017 and 2018 in Nebraska, USA. The PMGF to white popcorn was detected using a field corn pollen donor with yellow kernel color (dominate) and further confirmed with a molecular test. Popcorn kernels were harvested from cardinal and ordinal directions from 1 to 70 m. More than 7 million kernels were screened to detect the PMGF. Information-theoretic criteria were used to select the best-fit model. The greatest PMGF (1.6– 4.1%) was detected at 1 m and declined with distance. The PMGF was detected at 70 m, the maximum distance tested. Amplification of *Ga1* followed by restriction enzyme digest confirmed that yellow kernelled progeny were hybrids from the result of PMGF. This is the first assessment of PMGF from field corn to popcorn, and the results are alarming for the popcorn industry and export market.

1 | INTRODUCTION

Popcorn (*Zea mays* L. var. *everta*) is a popular healthy snack food in the United States and is increasing in popularity worldwide (Karababa, 2006; Lago et al., 2013). Popcorn is a recommended snack to meet daily ChooseMyPlate.gov whole grain consumption guidelines (USDA, 2015). This is because popcorn contains high fiber, no cholesterol, and low fat and several vitamins, minerals, and polyphenols (USDA-ARS, 2012, 2019). Popcorn is produced on nearly 90,000 ha annually in the United States (USDA-NASS, 2019). Popcorn is commonly grown in rotation with soybean [*Glycine max* (L.) Merr.]–field corn (*Zea mays* L. var. *indentata*) and produced under contracts with the private company or manufacturer

Abbreviations: GM, genetically modified; PCR, polymerase chain reaction; PMGF, pollen-mediated gene flow.

(D'Croz-Mason & Waldren, 1978; Ziegler, 2001). Field corn (also known as dent corn) accounts for >99% of all corn types grown in the United States and is used for ethanol, animal feed, human food, beverages, and industrial uses (USDA-NASS, 2019). Producers tend to financially benefit from the contract production of popcorn on their farms primarily in recent years where field corn, soybean, and wheat (*Triticum aestivum* L.) price is low (Edleman, 2004, 2006). The four largest popcorn-producing states in the United States are Nebraska, Indiana, Ohio, and Illinois. Additionally, abovementioned states are also among the eight largest field corn-producing states (USDA-NASS, 2019). Therefore, popcorn and field corn are grown in proximity with usually similar planting and flowering time. Whereas 92% of field corn planted in the United States is genetically modified (GM), there is no GM popcorn commercially available in the marketplace (ISAAA, 2019).

Popcorn relies on a gene for protection against pollen-mediated gene flow (PMGF) from field corn (Kermicle, Taba, & Evans, 2006; Ziegler, 2001). This gene is *gametophyte factor 1* (*ga1*) and mediates pollen–pistil interactions by encoding an enzyme that assists pollen tubes in growing within the silk body (style) (Moran Lauter, Muszynski, Huffman, & Scott, 2017). The *ga1* system is used elsewhere such as in organic production systems (Jones, Goodman, & Krakowsky, 2015). The *ga1* system is the only genetic system used for preventing gene flow from dent and sweet corn to popcorn (Jones & Goodman, 2016). The *ga1* locus has the *ga1*, *Ga1-s*, and *Ga1-m* alleles (Jimenez & Nelson, 1965; Jones & Goodman, 2016). The *ga1* allele is the most prevalent because it is carried by almost all commercially available field corn in the United States and has no barrier which allows it to be pollinated by all three alleles (Jones & Goodman, 2016; Nelson, 1993). The *Ga1-s* allele is used in popcorn breeding programs and commercial cultivation as it is nonreciprocal cross-sterile, which prevents pollination from *ga1* field corn, also known as dent sterility (Jones & Goodman, 2016; Kermicle & Evans, 2010; Zhang, Liu, Zhang, Jiang, & Cui, 2012). The *Ga1-m* allele is cross neutral, which means it can pollinate and accept pollen from any *gametophyte factor 1* allele including *Ga1-s* (Jimenez & Nelson, 1965; Jones & Goodman, 2016). *Ga1-m* is not prevalent in the United States but is being unintentionally introduced from Mexican and Central American germplasm to increase the genetic base of field corn (Jones & Goodman, 2016; Jones et al., 2015). Ten inbred lines have been released from North Carolina State University that unknowingly contained *Ga1-m* allele (Jones & Goodman, 2016; Jones et al., 2015). Jones and Goodman (2018) screened the maize nested association mapping population (NAM) and found 19 previously unreported *Ga1-m* alleles in these lines. A 2008 study concluded that 55% of commercial field corn hybrids planted in Mexican tropical, subtropical, and highlands were *Ga1-m* homozygous (De la Cruz Larios, González, Parra, Ruvalcaba, & Montes, 2008). Popcorn relies on *Ga1-s* to maintain genetic purity; however, the introduction of *Ga1-m* puts the popcorn production system at risk from field corn cross-pollination and genetic impurity that can affect popcorn export market (Jones & Goodman, 2016; Jones et al., 2015).

The major factors that contribute to corn genetic contamination are accidental seed impurity, sowing equipment and practices, cross-fertilization, volunteer plants, products mixing during harvest, transport, and storage processes (Devos et al., 2009). Pollen-mediated gene flow is the largest potential biological source of on farm mixing of genetic material in corn (Palaudelmàs et al., 2012). Pollen-mediated gene flow from genetically modified crops to non-genetically modified crops and organic crops (Jhala, Bhatt, Topinka, & Hall, 2011) or wild relatives (Ellstrand, 2001) is a concern. The PMGF in corn depends on a number of factors including, but not limited to, pollen viability, synchronization of flowering, and the relative concentrations of pollen in the donor and receptor fields (Della Porta et al., 2008; Duplessis & Dijkhuis, 1967; Messeguer et al., 2006). Additional factors such as wind direction and intensity, rainfall, and distance between the pollen source and pollen receptor are also important (Della Porta et al., 2008). An individual field corn tassel produces 2–5 million pollen grains (Goss, 1968; Uribelarrea, Carcova, Otegui, & Westgate, 2002). Additionally, pollen shed can last for 5–6 d and silks are receptive for ~5 d (Anderson, Lauer, Schoper, & Shibles, 2004; Westgate, Lizaso, & Batchelor, 2003). The second ear on a plant silks later than the first, increasing the timeframe that a field will have silks receptive to pollen (Van De Wiel & Lotz, 2006). Field corn pollen is ~100 μm compared with 17–58 μm in most other wind-pollinated plants (Stanley & Linskens, 2012). Despite pollen of field corn being relatively large and heavy, gene flow in corn has been shown at larger distances with favorable meteorological conditions (Kozjak, Sustar-Vozlic, & Meglic, 2011; Luna, Figueroa, Baltazar, Gomez, & Townsend, 2001; Messeguer et al., 2006). Isolation distances of 200 and 300 m are typically recommended to maintain 99 and 99.5% purity standards in field corn (Ingram, 2000; Luna et al., 2001; National Research Council, 2000); however, complete isolation of field corn fields planted at the same time requires 1,600 m (Bech, 2003).

Popcorn has a larger tassel that produces more pollen than field corn (Ziegler, 2001). Popcorn is prolific and generally produces two ears per plant but has smaller ear shoots than field corn (Ziegler, 2001). Isolation distance in popcorn is 200 m to avoid cross-contamination with other popcorn or *Ga1-s* specialty corn (Ziegler, 2001). Due to the presence of dent-sterility in popcorn, a physical isolation from field corn is not required to prevent cross-pollination

(Ziegler, 2001). However, field corn with the *Ga1-m* allele can cross-pollinate with popcorn, and genetic contamination of popcorn breeding or production programs from *Ga1-m* field corn would have significant impacts. Scientific information is not available in the literature about PMGF from field corn to popcorn. The objectives of this research were (a) to model the frequency of PMGF from a homozygous *Ga1-m* field corn to homozygous *Ga1-s* popcorn under field conditions, and (b) to evaluate the role of wild speed and direction. We hypothesized that PMGF from *Ga1-m* field corn to *Ga1-s* popcorn is possible and the frequency of gene flow will decrease with increasing distance from the pollen source.

2 | MATERIALS AND METHODS

2.1 | Plant materials and field experiments

The dent-sterile popcorn hybrid VWP111 (Conagra Brands), a white-kernelled popcorn hybrid, was selected as a female parent in this study. The *Ga1-m* homozygous field corn NC390 × NC394 (North Carolina State University), a yellow-kernelled F_2 inbred, was selected as a male parent. NC390 × NC394 is a hybrid known for abundant pollen production (Jones et al., 2015). Field experiments were conducted in 2017 and 2018 at the University of Nebraska–Lincoln's Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE (41.1704° N, 96.4615° W). The soil at the research site was a Yutan silty clay loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs) with a pH of 6.8 and 2.7% soil organic C. The experiments were under center pivot irrigation and were irrigated as needed. The experimental site was disked with a tandem disk to a depth of 10 cm and fertilized with 202 kg ha^{-1} of N in the form of anhydrous ammonia (82–0–0 N–P–K) in early spring. To achieve weed control, herbicide premixture atrazine–*S*-metolachlor (Bicep II Magnum, Syngenta Crop Protection) was applied at 2,470 g a.i. ha^{-1} preemergence after planting but before crop emergence, and postemergence at 2,470 g a.i. ha^{-1} when corn was at the three-leaf stage during both years.

A concentric donor–receptor design (i.e., Nelder wheel) was used for field experiments where the pollen donors were surrounded by pollen receptors (Jhala et al., 2011). The experiments were 120 × 120 m with a central square of 20 × 20 m for the pollen donor block and the entire outside square for the pollen receptor block. The field corn pollen donor (NC390 × NC394) and popcorn pollen receptor (VWP111, white popcorn hybrid) were planted at a constant density on 16 May 2017. In 2018, the field corn pollen donor was planted 11 d before the popcorn pollen receptor on 27 April and 8 May, respectively, both at a constant density. The receptor block was divided into eight directional blocks (cardinal: north, south, east, and west; ordinal: northeast, northwest, southeast, and southwest), and flags were placed at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, and 50 m in all eight directions and additionally at 60 and 70 m for ordinal directions.

2.2 | Flowering and seed harvest

The pollen donor and receptor blocks were monitored for beginning and ending pollen shedding and silking dates, and flowering synchrony was calculated as the percentage of days where donor block pollen shed and receptor block silking overlapped (Baltazar et al., 2015). At maturity, 60 ears from each direction and distance combination were hand harvested. Total kernels per treatment were determined by the average kernel number per ear of a six-ear subsample per treatment and multiplied by the total number of ears harvested in that treatment. Yellow kernels on white popcorn ears indicated that *Ga1-m*–mediated gene flow occurred. Yellow kernels per treatment were manually counted for the entire sample. The percentage of gene flow in each treatment was calculated by dividing the number of yellow kernels by the total kernels examined (Della Porta et al., 2008; Ma, Subedi, & Reid, 2004).

2.3 | Meteorological data

Meteorological data were recorded every 30 min (Della Porta et al., 2008) by installing a weather station (METER Group) at the experimental site that recorded half-hour averages of air temperature, relative humidity, wind speed, and wind direction. The weather station also recorded total precipitation and maximum wind gust speed for each half an hour. Data during days with flower synchrony were used for modeling PMGF.

2.4 | Statistical analysis

Gene flow frequency follows a binomial distribution with two possible outcomes in this study: yellow kernels (gene flow occurred) and white kernels (no gene flow occurred). The probability of gene flow is a function of the covariate, distance from pollen donor (Ganie & Jhala, 2017; Sarangi et al., 2017). Distance from the pollen donor can take on any real value; however, the probability of gene flow ranges between 0 and 1. Therefore, a logit function, or log-odds, must be used to transform the probability of gene flow data to remove the range and floor restrictions (Cramer, 2003):

$$\text{logit},\eta_I = \text{logit}\ (p_i) = \ln\left(\frac{p_i}{1+p_i}\right) \quad (1)$$

where η_I is the logit or log-odds link function, and p_i is the probability of success (yellow kernel), which ranges between 0 and 1. Data are back transformed for presentation.

A set of 564 possible models was constructed to explain the frequency of gene flow using an exponential decay function with distance from the pollen donor, direction of the pollen receptor relative to the pollen donor, average wind speed, wind frequency, wind direction, and/or maximum wind gust (Ganie & Jhala, 2017; Sarangi et al., 2017). Additionally, models were evaluated with and without thresholds of air temperature, relative humidity, and the dual threshold of air temperature and relative humidity. Models with air temperature thresholds considered gene flow only when temperatures were <35 °C. Temperatures >35 °C during anthesis have been shown to decrease pollen viability (Dupuis & Dumas, 1990; Herrero & Johnson, 1980; Schoper, Lambert, Vasilas, & Westgate, 1987). The maximum relative humidity was set at 75% because with relative humidity >75%, water forms a film on the pollen, causing it to clump (Di-Giovanni, Kevan, & Nasr, 1995; Knowlton, 1922). Models were evaluated considering both data from the entire 24 h during flower synchrony and during a 6-h time period between 0900 and 1500 h CST. The 6-h timeframe is when pollen shed typically occurs in corn (Della Porta et al., 2008).

2.5 | Model comparison and evaluation

Model comparison and selection was performed using the corrected Akaike's information criterion (AICc):

$$\text{AICc} = -2\text{LL} + 2K\,[n/(n-K-1)] \quad (2)$$

where K is the number of model parameters, LL is the maximum log likelihood, and n is the sample size (Anderson, 2007). The model with the greatest support is the is the one with the highest Akaike's information criterion weight (AICw):

$$\text{AICw}_i = \exp\left[\left(-\frac{1}{2\Delta i}\right) / \sum_{r=1}^{n} \exp\left(-\frac{1}{2\Delta r}\right)\right] \quad (3)$$

where Δi is the difference between the model with the lowest AIC and the ith model, r represents the total number of models being compared, and Δr is the difference between the model with the lowest AIC and the rth model. The model with the lowest AICc and the highest AICw is considered the best explanation of the data within the model set (Anderson, 2007). The best model was evaluated for goodness of fit using Pearson's χ^2 test (Ganie & Jhala, 2017; Sarangi et al., 2017).

2.6 | Model goodness of fit

Model goodness of fit was estimated using Pearson's χ^2 statistic:

$$\chi^2_{(n-k-1)} = \sum_i \frac{n_i(\gamma_i - \hat{\mu}_i)^2}{\hat{\mu}_i;(n_i - \hat{\mu}_i)} \quad (4)$$

where the sum of squared difference between the observed values (γ_i) and the fitted values for the ith group of observations ($\hat{\mu}_i$) is divided by the variance of γ_i that is $\mu_i(n_i - \hat{\mu}_i)/n_i$ (with μ_i estimated using $\hat{\mu}_i$), and n_i is the sample size for the ith group. The degrees of freedom for the χ^2 test are $n - k - 1$, where n refers to the total number of groups and k refers to the number of parameters (Ganie & Jhala, 2017; Sarangi et al., 2017).

2.7 | Molecular assay for gene flow confirmation

Two yellow corn kernels from each direction in each year at the farthest distance (50–70 m) and six kernels of the pollen donor and pollen receptor were planted in the greenhouse with 15 h of supplemental light, day temperature of 27–29 °C, and night temperature of 19–21 °C for 3 wk. DNA was extracted from ~150 mg of tissue using Quick DNA Plant/Seed Miniprep Kit (Zymo Research). The *Ga1-s* and *Ga1-m* coding sequence was amplified using forward and reverse primers (Table 1). Polymerase chain reaction (PCR) was performed with One Taq 2× Master Mix with standard buffer (New England Biolabs) using PCR settings of 30 s at 94 °C, followed by 30 s at 94 °C, 60 s at 55 °C, and 30 s at 68 °C repeated 29 times, followed by 5 min at 68 °C. Sanger sequencing of a cloned PCR product was performed to confirm that the PCR amplified the correct segment of the *Ga1* gene. A restriction enzyme digest was performed using Bs1I (New England Biolabs) for 8 min at 55 °C. Bs1I does not cut *Ga1-m* but does cut *Ga1-s* (Table 1). The products were analyzed via gel electrophoresis.

3 | RESULTS

3.1 | Flowering synchrony

In 2017, silking of the primary ear in the pollen receptor, *Ga1-s* popcorn, began 10 d before pollen shed of the pollen

TABLE 1 *Ga1-s* and *Ga1-m* gene sequences and primer sequences used for cloning of *Ga1-s* and *Ga1-m* from yellow-kernelled popcorn by dent corn hybrids listed in the 5′ to 3′ direction. Hybrids were from a field experiment conducted at the University of Nebraska–Lincoln, Eastern Nebraska Research and Education Center in 2017 and 2018

Genetic material	5′ to 3′ sequence
Ga1-s[a]	CACCGTGGACTTTGTGTTTGGCAATGCCCAGGCCATGTTCCAGAGCTGCGCGCTGCTGGT GCGCCGC**CCACCGAAAGG**CAAGCACAATGTGCTGACGGCCCAGGGCTGCAACAACGCAA GCCGCGAGTCCGGCTTCTCGTTCCACATGTGCACCGTGGAAGCCGCGCCGGGCGTGGACC TCGACGGCGTGGAGACCTACCTCGGCCGCCCCTACAGGAACTTCTCCCACGTCGCCTT CATCAAGTCGTATCTCAGTCGCGTGGTCA
Ga1-m[b]	CACCGTGGACTTTGTGTTTGGCAATGCCCAGGCCATGTTCCAGAGCTGCGCGCTGCTGGTGCG CCGCCCACCGA**ga**AAGGCAAGCACAATGTGCTGACGGCCCAGGGCTGCAACAACGCAAG CCGCGAGTCCGGCTTCTCGTTCCACATGTGCACCGTGGAAGCCGCGCCGGGCGTGGACC TCGACGGCGTGGAGACCTACCTCGGCCGCCCCTACAGGAACTTCTCCCACGTCGC CTTCATCAAGTCGTATCTCAGTCGCGTGGTCA
Forward primer	GCACCGTGGACTTTGTGTTTG
Reverse primer	CTGACCACGCGACTGAGATAC

[a] Bold text denotes the restriction enzyme cut site on *Ga1-s*

[b] Lowercase bold text denotes a 2-bp insertion that differentiates *Gal-m* from *Ga1-s*.

donor, *Ga1-m* field corn, began. The secondary popcorn ear silks were receptive to field corn pollen for 2 d before silking ceased, which accounted for 25–30% flowering synchrony in 2017. In 2018, overlap of *Ga1-m* field corn pollen shed and *Ga1-s* popcorn silking occurred for 7 d, ensuring 100% flowering synchrony.

3.2 | Meteorological data

Average daily temperatures and accumulated precipitation during the growing season followed the trend of the 30-yr average for the experimental site (Figure 1). During flowering synchrony, the average relative humidity and average air temperature (T) were 81% and 28 °C in 2017, respectively, and 82% and 24 °C in 2018, respectively (Table 2). When data during typical pollen shed hours (0900–1500 h CST) were considered, the average relative humidity was less (77–78%) and the average temperature was greater (Table 2).

Pearson correlation coefficients (r) showed a low degree of correlation ($r = 0 \leq .29$) between frequency of gene flow and wind parameters (average wind speed, wind frequency, and wind run in most cases; Table 3). This suggests that wind parameters contributed slightly to the frequency of PMGF in this study in any given direction. Correlation between frequency of gene flow and wind speed increased to a moderate degree ($r = .29 \leq .49$) up to 20 m from the pollen source when only data between 0900 and 1500 h CST was used. This suggests that the average wind speed during typical pollen shed hours contributes more towards frequency of gene flow than 24-h average wind speed.

The average wind speed at tassel height was 0.5 and 0.7 m s^{-1} during flowering synchrony in 2017 and 2018, respectively (Figure 2). Wind was predominately towards the north in 2017 and the north and southwest in 2018. The

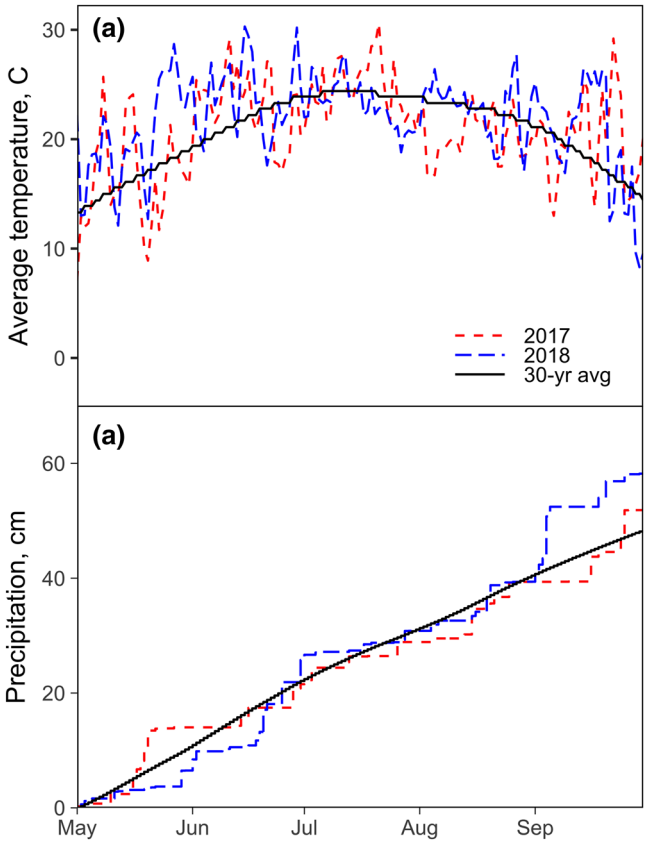

FIGURE 1 (a) Average daily air temperature and (b) precipitation during the 2017 and 2018 growing seasons and their 30-yr average at the University of Nebraska–Lincoln, Eastern Nebraska Research and Education Center near Mead, NE

TABLE 2 Average relative humidity and average air temperature observed during dent corn and popcorn pollination synchrony in a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018

Date	0900–1500 h CST		0000–2400 h CST	
	Relative humidity	Avg. air temperature	Relative humidity	Avg. air temperature
	%	°C	%	°C
21 July 2017	71	28	76	29
22 July 2017	85	31	85	27
2017 avg.	78	30	81	28
15 July 2018	69	30	77	25
16 July 2018	77	27	80	26
17 July 2018	91	21	89	22
18 July 2018	80	26	84	23
19 July 2018	78	28	82	25
20 July 2018	71	26	80	23
21 July 2018	73	27	80	23
2018 avg.	77	26	82	24

TABLE 3 Pearson's correlation coefficients between frequency of gene flow and wind parameters (wind speed, wind frequency, and wind run) during pollination from a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018

Timeframe[a]	Wind parameter	Distance from pollen source							All sampled distances
		1 m	2 m	5 m	10 m	15 m	20 m	50 m	
24 h	Wind speed	.26	.28	.30	.41	.41	.29	.12	.11
	Wind frequency	.13	−.27	−.19	.32	.24	.09	.30	.10
	Wind run	.27	.22	−.15	.38	.24	.15	.24	.08
6 h (0900–1500 h CST)	Wind speed	.41	.45	.37	.64	.63	.45	.17	.16
	Wind frequency	.22	.16	−.20	.28	.19	−.07	.34	.07
	Wind run	.28	.18	−.16	.42	.23	.04	.31	.08

[a] Data for wind parameters during flowering synchrony were tested during two timeframes; complete 24-h data and 6-h data collected between 0900 and 1500 h CST. The 6-h data represent typical pollen shed timeframe in dent corn.

air at tassel height was calm 46 and 21% of the time in 2017 and 2018, respectively.

3.3 | Frequency of pollen-mediated gene flow

A total of 5,980 popcorn ears were harvested, totaling>3.5 million kernels screened in 2017. Similarly, 6,240 popcorn ears were harvested in 2018, resulting in >3.6 million kernels screened (Table 4). Frequency of gene flow was highest at the closest distance (1 m) and declined quickly as distance from the pollen source increased (Figure 3). Regardless of direction from pollen source, frequency of gene flow at 1 m was .01615 (1.615%) and .04113 (4.113%) in 2017 and 2018, respectively. At the furthest distance (50 m) that all (eight) directions were sampled, the average frequency of gene flow was .00012 (0.012%) and .00058 (0.058%) in 2017 and 2018, respectively.

The PMGF varied between years and by direction from the pollen source. The inclusion of direction and year in the final model provided better estimation of PMGF than excluding direction and year based on the corrected Akaike information criterion (AICc). The PMGF followed a double exponential decay model where the first instance varied with year and the second instance varied with direction and year (Table 5). Pearson's X^2 test indicated a good fit of the model and the null hypothesis that the observed and predicted frequency of gene flow are the same; therefore, the null hypothesis cannot be rejected at $\alpha = .05$. The

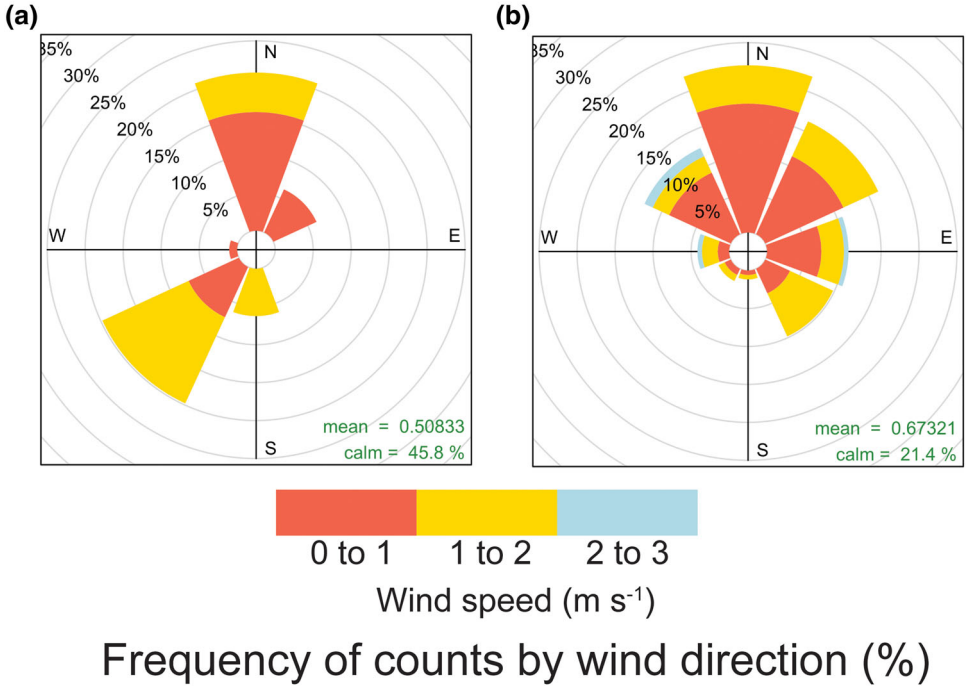

FIGURE 2 Windrose plots of the wind speed (m s^{-1}) and wind frequency (%) at tassel height during pollination synchrony in (a) 2017 and (b) 2018 at the University of Nebraska–Lincoln, Eastern Nebraska Research and Education Center near Mead, NE. Wind speed was low (0–3 m^{-2}) with frequent periods of calm wind in both years

distance where 90% reduction in gene flow occurred ranged from 6.2 to 16.8 m in 2017 and from 3.6 to 28.1 m in 2018 (Figure 3).

3.4 | Hybrid confirmation

The PMGF from *Ga1-m* field corn to *Ga1-s* popcorn was determined by kernel color. Amplification of *Ga1*, followed by restriction enzyme digest, confirmed that yellow-kernelled progeny were hybrids from the result of PMGF (Figure 4). The restriction enzyme, Bs1I, cut *Ga1-s* (two bands) but not *Ga1-m* (one band). The hybrids resulted in three bands: one from *Ga1-m* and two from *Ga1-s*.

4 | DISCUSSION

Flowering synchrony was 25–30% in 2017 when the pollen donor (yellow field corn) and pollen receptor (white popcorn) were planted on the same day. To increase flowering synchrony in 2018, the pollen donor was planted 11 d before the pollen receptor. This resulted in 100% flowering synchrony in 2018. The results indicate that maximum frequency of gene flow, .01615 (1.615%) and .04113 (4.113%), is observed at the closest distance of 1 m. Similar results were obtained in studies of gene flow from GM field corn to conventional field corn (Baltazar et al., 2015; Della Porta et al., 2008; Messeguer et al., 2006; Palaudelmàs et al., 2012). Baltazar et al. (2015) reported that the frequency of PMGF at 1 m ranged from .064 (6.4%) to .215 (21.5%) across eight field locations. The relative size of the pollen source to the pollen receptor increases the frequency of gene flow due to a greater relative area of pollen from the pollen source (Della Porta et al., 2008; Palaudelmàs et al., 2012). At 3.6–28.1 m, there was a 90% reduction in PMGF depending on year and direction. Pollen-mediated gene flow was detected at the maximum distance of 70 m tested in the ordinal directions.

A double exponential decay model was used to describe PMGF in this study. Beckie et al. (2011) used a double exponential decay model to explain PMGF in spring wheat. This approach for modeling PMGF has also been successful in weedy species such as barnyardgrass (*Echinochloa crus-gali* L.) (Bagavathiannan & Norsworthy, 2014), glyphosate-resistant to -susceptible waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] (Sarangi et al., 2017), interspecific hybridization of waterhemp and Palmer amaranth (*Amaranthus palmeri* S. Watson) (Oliveira et al., 2018), and glyphosate-resistant to -susceptible giant ragweed (*Ambrosia trifida* L.) (Ganie & Jhala, 2017).

Gene flow was affected by direction from the pollen source. Wind speed at tassel height was correlated with frequency of gene flow, but only to a low degree. Studies with greater correlation of wind parameters with frequency of gene flow in waterhemp and giant ragweed have been in

TABLE 4 Frequency of pollen-mediated gene flow from *Ga1-m* dent corn to *Ga1-s* popcorn in a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018

Year	Distance from pollen source	Kernels screened	Yellow kernels[a]	Frequency of gene flow[b]	Power $(1-\beta)$ $(\alpha = .05)$	
					$H_0 = 0.01$	$H_0 = 0.00001$
	m	——————no.——————				
2017	1	265,322	4,284	.01615	1	1
	2	270,593	940	.00347	1	1
	3	273,522	1,297	.00474	1	1
	4	272,936	1,835	.00672	1	1
	5	263,565	552	.00209	1	1
	10	268,251	531	.00198	1	1
	15	264,736	63	.00024	1	1
	20	268,251	38	.00014	1	0.99
	25	274,108	143	.00052	1	1
	30	283,479	34	.00012	1	0.99
	40	271,179	16	.00006	1	0.91
	50	262,979	32	.00012	1	0.99
	60	127,683	3	.00002	1	<0.80
	70	137,054	3	.00002	1	<0.80
	Total	3,503,657	9,771	.00279	1	1
2018	1	281,157	11,564	.04113	1	1
	2	281,222	4,328	.01539	1	1
	3	280,645	2,610	.00930	0.97	1
	4	280,584	2,789	.00994	<0.80	1
	5	281,212	1,856	.00660	1	1
	10	283,217	810	.00286	1	1
	15	281,739	324	.00115	1	1
	20	280,315	356	.00127	1	1
	25	284,091	125	.00044	1	1
	30	278,571	78	.00028	1	1
	40	285,714	40	.00014	1	0.99
	50	279,310	162	.00058	1	1
	60	143,750	23	.00016	1	0.99
	70	144,444	13	.00009	1	0.89
	Total	3,656,414	25,083	.00686	1	1

[a] Average pollen-mediated gene flow from all directions.
[b] Power values were calculated from a 95% confidence interval using binomial probabilities.

bare ground areas and report higher wind speeds (Ganie & Jhala, 2017; Sarangi et al., 2017), whereas, with a crop canopy present, wind speed tends to be slowed down (Baldocchi, Verma, & Rosenberg, 1983; Oliveira et al., 2018). Baltazar et al. (2015) reported a low degree of correlation between gene flow in field corn and wind speed ($X^2 = 0.01$), which indicates a low association between wind speed and gene flow frequency despite corn being a wind-pollinated species. Similarly, an incomplete association between wind direction and PMGF in field corn was observed by Della Porta et al. (2008). Ivanovska, Todorovski, Debeljak, and Džeroski (2009) reported a higher PMGF downwind when wind is blowing in a singular pronounced direction; however, there was no pattern in PMGF with wind blowing from distributed directions. In this study, wind direction did not dominate from a single direction.

Currently no isolation distance is required between popcorn and field corn. Field corn is used in border row as isolation in many popcorn breeding programs. With high- (4.113%) to low-level (0.002%) PMGF at 1 and 70 m, respectively from the pollen source, popcorn breeding programs and commercial production system are at major risk of

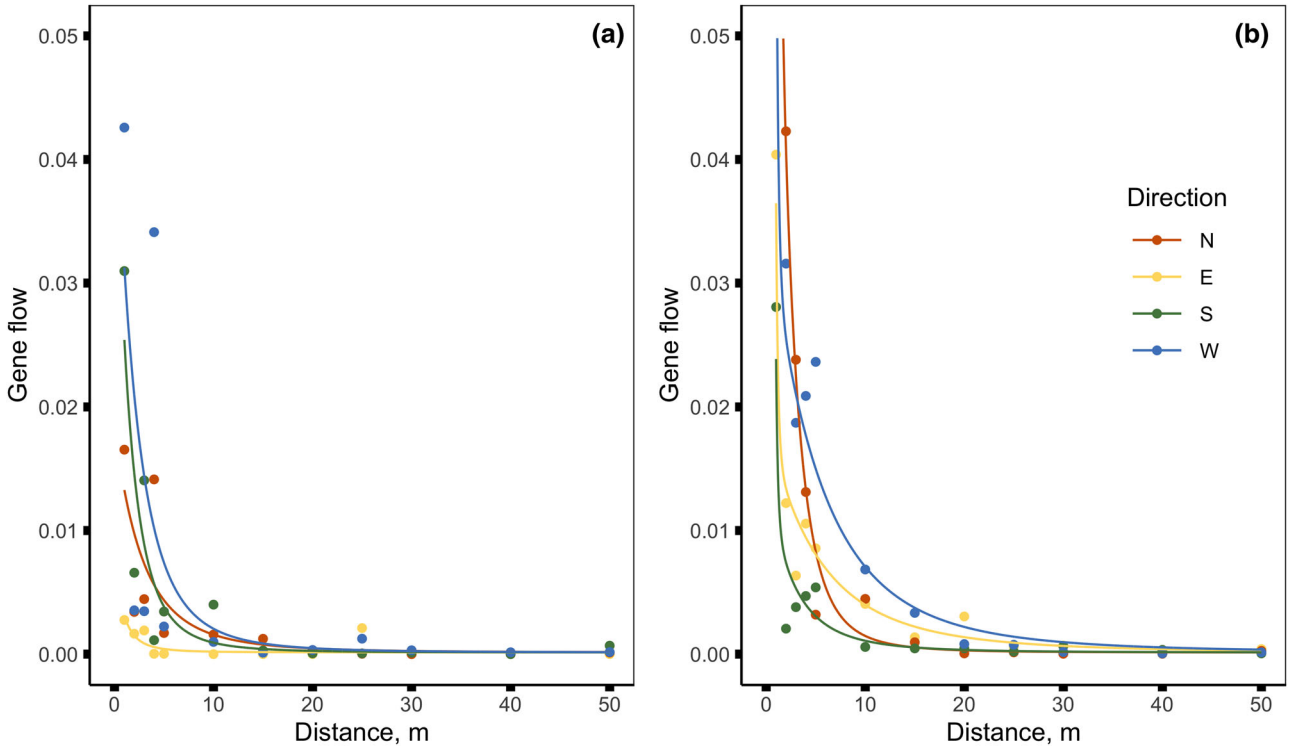

FIGURE 3 Pollen-mediated gene flow from (PMGF) *Ga1-m* field corn to *Ga1-s* popcorn as affected by distance from the pollen source in (a) 2017 and (b) 2018. The lines represent the best fit double exponential decay model where the first instance varied with year and the second instance varied with direction and year in each of the cardinal directions

Ga1-m field corn contamination. The most important issue for the maintenance of a cross-pollinated crop species in breeder production or seed production is genetic purity (Jones & Brooks, 1950). Contamination could go undetected for several years in a breeding program, as the majority of field corn and popcorn have yellow kernel color. Popcorn seed production is also at risk of field corn contamination. It is unlikely that contamination would be detected, and seed could be distributed to contract growers for production. The popcorn industry could be affected by PMGF that occurs during popcorn production if the seed is checked by country inspectors and samples return positive results of GM contamination. The European Union allows up to 0.9% GM contamination in approved food products if contamination is accidental and technically unavoidable but cannot be labeled as genetically modified organism (GMO) free above 0.1%. Currently there is no plan in place for the testing of current and pipeline commercial field corn hybrids for the presence of *Ga1-m* (Jones et al., 2015). This information would be beneficial for popcorn breeders and producers when communicating with neighbors or selecting bordering field corn hybrids. Jones et al. (2015) identified 10 sweet corn accessions derived from four landraces that showed *Ga1-m* resistance. These accessions could potentially be backcrossed into popcorn (Jones & Goodman, 2016). It is possible that PMGF in this study was underestimated because the size of the pollen donor block (field corn) was relatively small, producing less pollen to compete with the larger receptor block (popcorn) and because popcorn is a more prolific pollen producer than field corn.

Although many studies have investigated the role of distance, wind direction, and wind speed on PMGF in field corn, this work is the first to combine abovementioned factors and determine their overall importance on PMGF in popcorn. The popcorn industry should be cautious of potential *Ga1-m* field corn contamination. Known strategies to avoid PMGF in corn such as isolation distances, border rows harvested separately, using known *Ga1* field corn as border rows, and staggering planting dates to avoid flowering synchronization with neighboring field corn fields, should be used. Isolation distance is the most important factor for predicting PMGF; however, isolation distance is not currently used between field corn and popcorn. The implementation of isolation will not be a silver bullet for the popcorn industry, but because *Ga1-m* contamination even at a low rate could be detrimental, especially in a popcorn breeding program, it is the best option until *Ga1-m* resistance can be implemented.

TABLE 5 Estimation of coefficients, standard error, and test of significance for the double-exponential decay model[a] for the prediction of pollen-mediated gene flow from *Ga1-m* dent corn to *Ga1-s* popcorn from a field experiment conducted at the University of Nebraska–Lincoln Eastern Nebraska Research and Education Center in 2017 and 2018

Coefficients[b]	Estimate	SE	*z* value	Pr(>|*z*|)[c]
β_0	−8.88	0.10	−85.94	$<2\times10^{-16}$***
β_1	−1.65	3.68	−0.45	0.65
γ_1	−0.04	0.15	−0.30	0.77
β_1, Year 2	5.31	3.85	1.38	0.17
γ_1, Year 2	−3.77	1.31	−2.88	3.99×10^{-3}**
β_2	1.36	0.17	7.83	$<2\times10^{-16}$***
γ_2	−0.28	0.08	−3.70	2.19×10^{-4}***
β_2, north direction	0.19	0.06	3.26	1.13×10^{-3}**
β_2, northeast direction	−0.03	0.08	−0.34	0.74
β_2, northwest direction	0.15	0.06	2.55	0.01*
β_2, south direction	0.38	0.07	5.58	0.00***
β_2, southeast direction	−0.21	0.10	−2.10	0.04*
β_2, southwest direction	0.06	0.06	1.02	0.31
β_2, west direction	0.38	0.07	5.70	1.22×10^{-8}***
γ_2, north direction	0.21	0.08	2.73	0.01**
γ_2, northeast direction	0.05	0.05	0.95	0.34
γ_2, northwest direction	0.21	0.08	2.69	0.01**
γ_2, south direction	0.17	0.07	2.33	0.02*
γ_2, southeast direction	0.19	0.07	2.54	0.01*
γ_2, southwest direction	0.21	0.08	2.72	0.01**
γ_2, west direction	0.20	0.08	2.67	0.01**
β_2, Year 2	0.24	0.17	1.42	0.16
γ_2, Year 2	0.24	0.08	3.21	1.35×10^{-3}**
β_2, north direction, Year 2	0.17	0.06	2.84	4.51×10^{-3}**
β_2, northeast direction, Year 2	−0.43	0.08	−5.36	8.55×10^{-8}***
β_2, northwest direction, Year 2	−0.03	0.06	−0.53	0.60
β_2, south direction, Year 2	−0.43	0.07	−6.27	3.57×10^{-10}***
β_2, southeast direction, Year 2	0.16	0.10	1.54	0.12
β_2, southwest direction, Year 2	−0.18	0.06	−2.79	0.01**
β_2, west direction, Year 2	−0.26	0.07	−3.80	1.48×10^{-4}***
γ_2, north direction, Year 2	−0.28	0.08	−3.64	2.77×10^{-4}***
γ_2, northeast direction, Year 2	−0.03	0.05	−0.65	0.52
γ_2, northwest direction, Year 2	−0.24	0.08	−3.07	2.14×10^{-3}**
γ_2, south direction, Year 2	−0.21	0.07	−2.93	3.42×10^{-3}**
γ_2, southeast direction, Year 2	−0.19	0.07	−2.62	0.01**
γ_2, southwest direction, Year 2	−0.23	0.08	−2.93	3.44×10^{-3}**
γ_2, west direction, Year 2	−0.20	0.08	−2.63	0.01**

[a] $\text{logit}(\rho_i) = \beta_0 + \exp[\beta_1(\text{Year}) + \gamma_1(\text{Year}) \times \text{Distance}] + \exp[\beta_2(\text{Direction:Year}) + \gamma_2(\text{Direction:Year}) \times \text{Distance}]$, where ρ_i is frequency of gene flow of the ith observation; β_0 is the overall intercept; β_1 and β_2 are the intercepts for the first and second instances, respectively; and γ_1, and γ_2 are the decay rates.

[b] β_2 and γ_2 vary with the direction and the year.

*Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level.

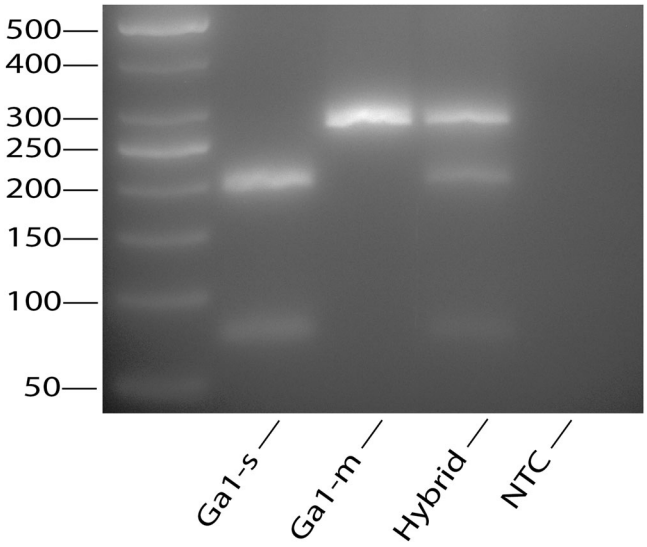

FIGURE 4 Polymerase chain reaction (PCR) amplification of the *Ga1* coding sequence was performed for the *Ga1-s* and *Ga1-m* parents and hybrid (*Ga1-s* × *Ga1-m*). A restriction enzyme digest was performed using Bs1I, which does not cut *Ga1-m* (one band) but does cut *Ga1-s* (two bands). Gene flow from *Ga1-m* field corn to *Ga1-s* popcorn results in hybrids, and restriction enzyme cuts half the PCR product (three bands). NTC, nontreated control

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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